UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, DC 20460

OFFICE OF CHEMICAL
SAFETY AND POLLUTION
PREVENTION

April 10, 2015

MEMORANDUM

Subject: Efficacy Review for Synergex, EPA File Symbol 1677-ELN; DB Barcode: D424062.

From: Ibrahim Laniyan, Ph.D.
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Applicant: Ecolab, Inc.
370 N. Wabasha Street
St. Paul, MN 55102

Formulation from the Label:

Active Ingredients	% by wt.
Hydrogen Peroxide	10.70 %
Peroxyoctanoic Acid	0.63 %
Peroxyacetic Acid	2.38 %
Other Ingredients	86.29 %
Total	100.00 %

I. BACKGROUND

The product, Synergex (EPA File Symbol 1677-ELN), is a new product. The applicant requested to register the product for use as a food and non-food contact sanitizer. Studies were conducted at ATS Labs, located at 1285 Corporate Center Drive, Suite 110, Eagan, MN 55121; Ecolab, located at Ecolab Schuman Campus, 655 Lone Oak Drive, Eagan, MN 55121-1560.

This data package identified as D424062 contained a letter from the applicant's representative to EPA (dated October 20, 2014) EPA Form 8570-1 (Application for Pesticide), EPA Form 8570-4 (Confidential Statement of Formula), EPA Form 8570-34 (Certification with Respect to Citation of Data), EPA Form 8570-35 (Data Matrix), eighteen studies (MRID Nos. 494674-03 through 494674-20), Statements of No Data Confidentiality for all eighteen studies, and the proposed label (dated October 20, 2014).

II. USE DIRECTIONS

SANITIZATION
Synergex acid sanitizer is recommended for use on pre-cleaned, hard, non-porous surfaces in food and beverage processing, industrial, and institutional applications. This product is effective as a sanitizer when solution is prepared in water of up to 500 ppm hardness as $CaCO_3$.
NOTE: FOR MECHANICAL OPERATIONS prepared use solution may not be reused for sanitizing but may be reused for other purposes such as cleaning.
FOR MANUAL OPERATIONS fresh sanitizing solutions must be prepared at least daily or more often if the solution becomes diluted or soiled.

Sanitizing Food Contact Surfaces
Prior to sanitizing, remove gross food particles, and then wash with a detergent solution, followed by a potable water rinse. Sanitize with a concentration of 1 ounce Synergex concentrate per 4 – 7 gallons of water (0.112-0.195% v/v or 1120 ppm –1950 ppm product or 1.12-1.95 ml/L). Use immersion, coarse spray or circulation techniques as appropriate to the equipment. All surfaces must be exposed to the sanitizing solution for a period of not less than 1 minute unless a longer time is specified by the governing sanitary code. Drain thoroughly and allow to dry. Do not rinse.

Sanitizing Hard, Non-Porous, Non-Food Contact Surfaces
Prior to use of this product, remove gross soil particles from surfaces. Wash with a detergent solution and rinse with potable water. Sanitize hard, non-porous, non-food contact surfaces such as floors, walls, tables, chairs, benches, drains, troughs, and drip pans with 1 ounce of Synergex concentrate per 4 – 8 gallons of water (0.098-0.195% v/v or 980 ppm–1950 ppm product or 0.98 ml/L-1.95 ml/L). Apply use solution using a cloth, mop, sponge, coarse sprayer, or by immersion. All surfaces must be exposed to the sanitizing solution for a period of not less than 5 minutes. Drain thoroughly and allow to air dry. No rinse necessary.

Foam Sanitizing Non-Food Contact Surfaces (This use not approved in the state of California)
Synergex in conjunction with Liquid K is an effective foam sanitizer of pre-cleaned non-food contact surfaces, such as floors, walls, drains, and equipment surfaces. For this application, prepare a solution of 1 ounce of Synergex concentrate per 4 – 8 gallons of water (0.098-0.1950% v/v or 980ppm – 1950 ppm product or 0.98-1.95ml/L) and 1 ounce to 2 ounces Liquid K per 6 gallons water (0.13% – 0.26% v/v). For example, in 6 gallons of water, add 1 ounce of Synergex and 1 -2 ounces of Liquid K**.** Liquid K is the only approved foam generator. Apply solution as a foam using recommended equipment. Wet surfaces thoroughly with foam. Surfaces must be exposed to the sanitizing foam for a period of not less than 5 minutes. Drain thoroughly and allow to air dry. No rinse is necessary. Contact your Ecolab representative for information on Liquid K foaming agents and recommended foaming equipment.

DISINFECTION

Synergex disinfects as it cleans in one operation. Synergex can be used to disinfect floors, walls and other hard, non-porous, non-food contact surfaces such as tables, chairs, countertops, bathroom fixtures, sinks, shelves, racks, carts, refrigerators, coolers, glazed tile, linoleum, vinyl, glazed porcelain, plastic (such as polypropylene and polyethylene), stainless steel, or glass.

Areas of use: Use Synergex in veterinary clinics, animal life science laboratories, industrial facilities, office buildings, recreational facilities, retail and wholesale establishments.

Combination Disinfection and Cleaning

Synergex is effective as a disinfectant at a concentration of 1 – 7.68 ounces Synergex concentrate per 3 gallons (0.260 – 2% v/v concentration or 2600-20000 ppm product or 2.6-20ml/L) of hard water (500 ppm as $CaCO_3$), and 5% blood serum on hard non-porous surfaces. At this dilution, Synergex is effective against *Staphylococcus aureus* (ATCC 6538)*, Salmonella enterica (*ATCC 10708) and *Pseudomonas aeruginosa* (ATCC 15442).

For heavily soiled areas a pre-cleaning step is required. Apply solution with mop, cloth, sponge, brush, scrubber, or coarse spray device or by soaking so as to wet all surfaces thoroughly. All surfaces must remain wet for 10 minutes, and then remove solution and entrapped soil with a clean wet mop, cloth, or wet vacuum pickup. Prepare a fresh solution daily or when it becomes soiled or diluted. Rinse food contact surfaces that come in contact with food with a potable water rinse prior to reuse.

Disinfecting Hard Non-Porous and Non-Food Contact Surfaces Synergex is recommended for use on hard, non-porous, environmental surfaces such as floors, walls and processing equipment. Synergex is effective against *Staphylococcus aureus* (ATCC 6538)*, Salmonella enterica* (ATCC 10708) and *Pseudomonas aeruginosa* (ATCC 15442) at a concentration of 1 – 7.68 ounces Synergex concentrate per 3 gallons (0.260 – 2% v/v concentration or 2600-20000 ppm product or 2.6-20ml/L) of hard water (500 ppm as $CaCO_3$) and 5% blood serum. For heavily soiled areas a pre-cleaning step is required. Rinse all surfaces thoroughly with the disinfecting solution and maintain a contact time of at least 10 minutes. Product contact surfaces must be rinsed with sterile water.

Virucidal

At 1 ounce of Synergex concentrate per 4 – 7 gallons of water (0.112-0.195% v/v or 1120ppm – 1950ppm product or 1.12–1.95ml/L) Synergex is effective against Influenza B (ATCC VR-823), Influenza A (H1N1) (ATCC VR-897), and Reovirus (ATCC VR-232) on hard inanimate surfaces when used at a 5 minute contact time in the presence of 500 ppm hard water and 5% blood serum. Apply as directed under disinfection.

Fungicidal

Synergex can be used on hard non-porous inanimate surfaces such as shower room floors, locker room benches, shower stalls and bath mats. At 1 ounce of Synergex concentrate per 4 – 5.5 gallons of water (0.142-0.195% v/v or 1420ppm – 1950ppm product or 1.42–1.95ml/L) Synergex is effective against *Trichophyton mentagrophytes* (Athletes Foot Fungi) (ATCC 9533) in the presence of protein (5% blood serum) in 500 ppm hard water with a 10 minute contact time. Apply as directed under disinfection.

III. AGENCY STANDARDS FOR PROPOSED CLAIMS

Disinfectants for Use on Hard Surfaces in Hospital or Medical Environments: The effectiveness of disinfectants for use on hard surfaces in hospital or medical environments must be substantiated by data derived using the AOAC Use-Dilution Method (for water soluble powders and liquid products) or the AOAC Germicidal Spray Products Test (for spray products), or the AOAC Hard Surface Carrier Test. The tests require that sixty carriers must be tested with each of

3 samples, representing 3 different product lots at the LCL, against *Staphylococcus aureus* ATCC 6538 (for effectiveness against Gram-positive bacteria), and *Pseudomonas aeruginosa* ATCC 15442 (representative of a nosocomial pathogen), [120 carriers per sample; a total of 360 carriers]. To support products labeled as "disinfectants", killing on 59 out of 60 carriers is required in AOAC Germicidal Spray Products Test to provide effectiveness at the 95% confidence level. To pass performance requirements when using AOAC Hard Surface Carrier Test, tests must result in killing in 58 out of each set of 60 carriers for *Staphylococcus aureus* ATCC 6538; 57 out of each set of 60 carriers for *Pseudomonas aeruginosa* ATCC 15442. Performance requirements when using AOAC Use-Dilution Method are killing in 57 out of each set of 60 carriers for *Staphylococcus aureus* ATCC 6538 and 54 out of each set of 60 carriers for *Pseudomonas aeruginosa* ATCC 15442. Each microbe should be tested three times. Each test should be conducted against a separate batch of product for a total of three batches. Each of the three tests should be conducted on a different day.

Disinfectants for Use as Fungicides (Against Pathogenic Fungi, Using a Modified Method): The effectiveness of liquid disinfectants against specific pathogenic fungi must be supported by efficacy data using an appropriate test. The AOAC Use-Dilution Method (for water soluble powders and liquid products) or the AOAC Germicidal Spray Products as Disinfectants Method (for spray products) may be modified to conform with the appropriate elements in the AOAC Fungicidal Test. The inoculum in the test must be modified to provide a concentration of at least 10^6 conidia per carrier. Ten carriers on each of 2 product samples at LCL representing 2 different product lots must be employed in the test. Killing of the specific pathogenic fungi on all carriers is required.

Virucides: The effectiveness of virucides against specific viruses must be supported by efficacy data that simulates, to the extent possible in the laboratory, the conditions under which the product is intended to be used. Carrier methods that are modifications of either the AOAC Use-Dilution Method (for liquid disinfectants) or the AOAC Germicidal Spray Products as Disinfectants Method (for spray disinfectants) must be used. To simulate in-use conditions, the specific virus to be treated must be inoculated onto hard surfaces, allowed to dry, and then treated with the product according to the directions for use on the product label. One surface for each of 2 different product lots of disinfectant at LCL must be tested against a recoverable virus titer of at least 10^4 from the test surface for a specified exposure period at room temperature. Then, the virus must be assayed by an appropriate virological technique, using a minimum of four determinations per each dilution assayed. Separate studies are required for each virus. The calculated viral titers must be reported with the test results. For the data to be considered acceptable, results must demonstrate complete inactivation of the virus at all dilutions. When cytotoxicity is evident, at least a 3-log reduction in titer must be demonstrated beyond the cytotoxic level.

Sanitizer Test (for inanimate, non-food contact surfaces): The effectiveness of sanitizers for non-food contact surfaces must be supported by data that show that the product will substantially reduce the numbers of test bacteria on a treated surface over those on an untreated control surface. The test surface(s) should represent the type(s) of surfaces recommended for treatment on the label, i.e., porous or non-porous. Products that are represented as "one-step sanitizers" should be tested with an appropriate organic soil load, such as 5 percent serum. Tests should be performed with each of 3 product samples, representing 3 different product lots at the LCL against *Staphylococcus aureus* (ATCC 6538) and either *Klebsiella pneumoniae* (aberrant, ATCC 4352) or *Enterobacter aerogenes* (ATCC 13048 or 15038). The ASTM method states that the inoculum employed should provide a count of at least 7.5×10^5 colony forming units per carrier. Results must show a bacterial reduction of at least 99.9 percent over the parallel control within 5 minutes.

Sanitizers (For Non-Food Contact Surfaces; Additional Bacteria): There are cases where an applicant requests to make claims of effectiveness against additional microorganisms for a product that is to be used as a sanitizer for non-food contact surfaces. Confirmatory test standards would apply. Therefore, 2 product samples, representing 2 different product lots at LCL, should be tested against each additional microorganism. The ASTM method states that the inoculum employed should provide a count of at least 7.5 x 10^5 colony forming units per carrier. Results must show a bacterial reduction of at least 99.9 percent over the parallel control within 5 minutes.

Sanitizing Rinses (For Previously Cleaned, Food Contact Surfaces): Sanitizing rinses may be formulated with quaternary ammonium compounds, chlorinated trisodium phosphate, or anionic detergent-acid formulations. The effectiveness of such sanitizing rinses for previously cleaned, food contact surfaces must be substantiated by data derived from the AOAC Germicidal and Detergent Sanitizing Action of Disinfectants Method. Data from the test on 1 sample from each of 3 different product lots at the LCL, against *Escherichia coli* (ATCC 11229) and *Staphylococcus aureus* (ATCC 6538) are required. When the effectiveness of the product in hard water is made, all required data must be developed at the hard water tolerance claimed. Results must demonstrate a 99.999% reduction in the number of microorganisms within 30 seconds. The results must be reported according to the actual count and the percentage reduction over the control. Furthermore, counts on the number controls for the product should fall between 75 and 125 x 10^6/ml for percent reductions to be considered valid. Label directions for use must state that a contact time of at least 1 minute is required for sanitization. A potable water rinse is not required (to remove the use solution from the treated surface) for products cleared for use on food contact surfaces under the Federal Food, Drug, and Cosmetic Act. Label directions must recommend a potable water rinse (to remove the use solution from the treated surface) under any other circumstances. These Agency standards are presented in OCSPP 810.2300, as well as the AOAC Germicidal and Detergent Sanitizing Action of Disinfectants Method.

Sanitizing Rinses for Previously Cleaned Food Contact Surfaces – Additional Microorganisms: There are cases where an applicant requests to make claims of effectiveness against additional microorganisms for a product that is already registered as a sanitizing rinse for previously cleaned food contact surfaces. Confirmatory test standards would apply. For sanitizing rinses for previously cleaned food contact surfaces, 2 product samples, representing 2 different batches at the LCL, must be tested against each additional microorganism. Results must demonstrate a 99.999% reduction in the number of microorganisms within 30 seconds. The results must be reported according to the actual count and percentage reduction over the control. Furthermore, according to information in the above AOAC test method itself, counts on the numbers control for the product should fall between 75 and 125 x 10^6/ml for percent reductions to be considered valid. The minimum concentration of the product which provides the results required above is the minimum effective concentration. Label directions for use, however, must state that a contact time of at least 1 minute is required for sanitization. The above Agency standards are presented in OCSPP 810.2300, as well as the AOAC Germicidal and Detergent Sanitizing Action of Disinfectants Method.

IV. BRIEF DESCRIPTION OF THE DATA

Note: The product lots P072241, P081931, P111431, and P120331 were diluted to the LCL using 500 ppm AOAC synthetic hard water.

1. MRID 494674-03 "Germicidal and Detergent Sanitizing Action of Disinfectants, Test Organisms: *Escherichia* coli O157:H7 (ATCC 35150), *Listeria monocytogenes*

(ATCC 49594), and *Salmonella enterica* subsp. *enterica* Typhimurium (ATCC 13311)"; by Joshua Luedtke. Study conducted at ATS Labs. Study completion date – July 10, 2014. Study Number A16705; Ecolab GLP Study Number: 1300150CL1.

This study was conducted against *Escherichia* coli O157:H7 (ATCC 35150), *Listeria monocytogenes* (ATCC 49594), and *Salmonella enterica* subsp. *enterica* Typhimurium (ATCC 13311). Two lots (Lot Nos. P111431-2 and P120331-2) of the product, KX-6228 (Synergex), were tested using the ATS Labs protocol ECO01042914.GDST.1 (copy provided). A use solution was prepared by adding 1.19 g test substance to 998.81 g of 500 ppm AOAC synthetic hard water (titrated at 503 ppm; a 1 oz. per 7 gallons dilution). A 99-ml aliquot of the prepared use solution was transferred to each of 250-300 ml Erlenmeyer flasks. The flasks were placed in a 25±1°C water bath for at least 10 minutes. One-ml adjusted bacterial suspension was added to each flask. One-ml aliquots of the bacterium-test solution were transferred to 9 ml of Neutralizer 30 seconds after the addition of the bacterial suspension. After vortex mixing, four 1.0 ml and four 0.1 ml aliquots of the neutralized material were spread-plated onto the subculture agar medium. All subculture plates were incubated for 24-30 hours at 35-37°C. Following incubation, the subculture plates were visually examined for growth. Representative test and positive control subcultures showing growth were visually examined, Gram stained and biochemically assayed to confirm or rule out the presence of the test organism. Controls included purity, sterility, viability, numbers control, and neutralization confirmation. The reported average initial colony forming units per ml, for each test microorganism, are as follows: ***Escherichia* coli O157:H7 (3.0×10^7, $7.48\log_{10}$), *Listeria monocytogenes* (7.9×10^7, $7.9\log_{10}$), and *Salmonella enterica* subsp. *enterica* Typhimurium (9.5×10^7, $7.98\log_{10}$)**.

2. MRID 494674-04 "Germicidal and Detergent Sanitizing Action of Disinfectants, Test Organisms: *Campylobacter jejuni* (ATCC 29428)"; by Joshua Luedtke. Study conducted at ATS Labs. Study completion date – July 2, 2014. Study Number A16706; Ecolab GLP Study Number: 1300150CL2.

This study was conducted against *Campylobacter jejuni* (ATCC 29428). Two lots (Lot Nos. P111431-2 and P120331-2) of the product, KX-6228 (Synergex), were tested using the ATS Labs protocol ECO01042914.GDST.2 (copy provided). A use solution was prepared by adding 1.19 g test substance to 998.81 g of 500 ppm AOAC synthetic hard water (titrated at 517 ppm; a 1 oz. per 7 gallons dilution). A 99-ml aliquot of the prepared use solution was transferred to each of 250-300 ml Erlenmeyer flasks. The flasks were placed in a 25±1°C water bath for at least 10 minutes. One-ml adjusted bacterial suspension was added to each flask. One-ml aliquots of the bacterium-test solution were transferred to 9 ml of Neutralizer 30 seconds after the addition of the bacterial suspension. After vortex mixing, four 1.0 ml and four 0.1 ml aliquots of the neutralized material were spread-plated onto the subculture agar medium. All subculture plates were incubated for 4 days at 35-37°C. Following incubation, the subculture plates were visually examined for growth. Representative test and positive control subcultures showing growth were visually examined, Gram stained and biochemically assayed to confirm or rule out the presence of the test organism. Controls included purity, sterility, viability, numbers control, and neutralization confirmation. The reported average initial colony forming units per ml, for each test microorganism, are as follows: ***Campylobacter jejuni* (7.4×10^7, $7.87\log_{10}$)**.

3. MRID 494674-05 "Germicidal and Detergent Sanitizing Action of Disinfectants, Test Organisms: *Cronobacter sakazakii* (ATCC 12868) and *Pseudomonas aeruginosa* (ATCC 15442)"; by Joshua Luedtke. Study conducted at ATS Labs. Study completion date – July 2, 2014. Study Number A16707; Ecolab GLP Study Number: 1300150CL3.

This study was conducted against *Cronobacter sakazakii* (ATCC 12868) and *Pseudomonas aeruginosa* (ATCC 15442). Two lots (Lot Nos. P111431-2 and P120331-2) of the product, KX-6228 (Synergex), were tested using the ATS Labs protocol ECO01042914.GDST.3 (copy provided). A use solution was prepared by adding 1.19 g test substance to 998.81 g of 500 ppm AOAC synthetic hard water (titrated at 503 ppm; a 1 oz. per 7 gallons dilution). A 99-ml aliquot of the prepared use solution was transferred to each of 250-300 ml Erlenmeyer flasks. The flasks were placed in a 25±1°C water bath for at least 10 minutes. One-ml adjusted bacterial suspension was added to each flask. One-ml aliquots of the bacterium-test solution were transferred to 9 ml of Neutralizer 30 seconds after the addition of the bacterial suspension. After vortex mixing, four 1.0 ml and four 0.1 ml aliquots of the neutralized material were spread-plated onto the subculture agar medium. All Pseudomonas *aeruginosa* subculture plates were incubated for 24-30 hours at 35-37°C. All *Cronobacter sakazakii* subculture plates were incubated for 24-30 hours at 25-30°C. Following incubation, the subculture plates were visually examined for growth. Representative test and positive control subcultures showing growth were visually examined, Gram stained and biochemically assayed to confirm or rule out the presence of the test organism. Controls included purity, sterility, viability, numbers control, and neutralization confirmation. The reported average initial colony forming units per ml, for each test microorganism, are as follows: ***Cronobacter sakazakii*** **(4.9×10^7, $7.69 \log_{10}$) and *Pseudomonas aeruginosa* (3.9×10^7, $7.59 \log_{10}$)**

Note: Protocol amendment reported in the study was reviewed.

4. MRID 493674-06 "Standard Test Method for Efficacy of Sanitizers Recommended for Inanimate Non-Food Contact Surfaces (Dilutable), Test Organisms: *Escherichia coli* O157:H7 (ATCC 35150) and *Listeria monocytogenes* (ATCC 49594)"; by Kristen Niehaus. Study conducted at ATS Labs. Study completion date – September 22, 2014. Study Number A16708; Ecolab GLP Study Number: 1300150CL4.

This study was conducted against *Escherichia coli* O157:H7 (ATCC 35150) and *Listeria monocytogenes* ATCC 49594). Four lots (Lot Nos. P111431-2, P120331-2, P111431-CL, and P120331-CL) of the product, KX-6228 (Synergex), were tested using the ATS Labs protocol ECO01050114.NFS.1 (copy provided). A use solution was prepared by adding 1.56 g test substance to 1498.44 g of 500 ppm AOAC synthetic hard water (titrated at 500 ppm; a 1 oz. per 8 gallons dilution). Fetal bovine serum was added to each inoculum to achieve a 5% organic soil load. Five sterile stainless steel carriers per product lot per organism were inoculated with 0.02 ml (*E. coli*) or 0.03 ml (*L. monocytogenes*) of a 48-54 hour old suspension of the test organism. The inoculum was spread to within 1/8 inch of the edges of the carrier. The carriers were dried at 35-37°C and a relative humidity of 40% for 35 minutes with the Petri dish lids intact (*E. coli*) or 20 minutes with the Petri dish lids slightly ajar (*L. monocytogenes*). Each carrier was transferred to a sterile jar and was exposed to 5.0 ml of the use solution at 20.9°C (*E. coli*) or 22.9°C (*L. monocytogenes*) for 5 minutes. After exposure, 20 ml of D/E Neutralizing Broth was added to each jar and the jars were rotated vigorously to suspend the surviving organisms. Within 30 minutes of the addition of the neutralizer, 1.0 ml aliquots of the 10^0 dilutions were plated in duplicate on Tryptic Soy Agar with 5% Sheep Blood. The plates were incubated at 35-37°C for 48±4 hours. The *Listeria monocytogenes* subcultures were placed at 2-8°C for 2 days prior to examination. Following incubation and storage, the subcultures were visually enumerated. Controls included those for carrier quantitation, inoculum count, viability, neutralization confirmation, sterility, and purity. The reported average colony forming units (CFU) per control carrier, for each test microorganism, are as follows: ***Escherichia coli*** **O157:H7 3.24×10^6 ($6.51 \log_{10}$) and *Listeria monocytogenes* 1.78×10^6 ($6.25 \log_{10}$)**.

Note: Protocol amendments reported in the study were reviewed.

Note: The applicant provided the data for two failed trials. In those trial, the numbers controls were below the required number. Thus, the tests were invalid. These data were not used to evaluate efficacy of the test product. See Attachment I of the laboratory report.

5. MRID 493674-07 "Standard Test Method for Efficacy of Sanitizers Recommended for Inanimate Non-Food Contact Surfaces (Dilutable), Test Organisms: *Cronobacter sakazakii* (ATCC 12868) and *Salmonella enterica* subsp. *enterica* Typhimurium (ATCC 13311)"; by Kristen Niehaus. Study conducted at ATS Labs. Study completion date – July 15, 2014. Study Number A16709; Ecolab GLP Study Number: 1300150CL5.

This study was conducted against *Cronobacter sakazakii* (ATCC 12868) and *Salmonella enterica* subsp. *enterica* Typhimurium (ATCC 13311). Two lots (Lot Nos. P111431-2 and P120331-2) of the product, KX-6228 (Synergex), were tested using the ATS Labs protocol ECO01050114.NFS.2 (copy provided). A use solution was prepared by adding 1.56 g test substance to 1498.44 g of 500 ppm AOAC synthetic hard water (titrated at 503 ppm; a 1 oz. per 8 gallons dilution). Fetal bovine serum was added to each inoculum to achieve a 5% organic soil load. Five sterile stainless steel carriers per product lot per organism were inoculated with 0.02 ml of a 48-54 hour old suspension of the test organism. The inoculum was spread to within 1/8 inch of the edges of the carrier. The carriers were dried at 36.2°C and a relative humidity of 40% for 40 minutes with the Petri dish lids slightly ajar. Each carrier was transferred to a sterile jar and was exposed to 5.0 ml of the use solution at 21.6°C and 53.8% relative humidity for 5 minutes. After exposure, 20 ml of D/E Neutralizing Broth was added to each jar and the jars were rotated vigorously to suspend the surviving organisms. Within 30 minutes of neutralization, duplicate 1.00 ml and 0.100 ml aliquots of the neutralized solution (10°) were plated onto the recovery agar plate medium. The S. *enterica* plates were incubated at 35-37°C for 48±4 hours. The *C. sakazakii* plates were incubated for 48±4 hours at 25-30°C. Following incubation the subcultures were visually enumerated. Controls included those for carrier quantitation, inoculum count, viability, neutralization confirmation, sterility, and purity. The reported average colony forming units (CFU) per control carrier, for each test microorganism, are as follows: ***Cronobacter sakazakii* O157:H7 6.03 x 10^4 (4.78$\log_{10}$) and *Salmonella enterica* subsp. *enterica* Typhimurium 5.37 x 10^5 (5.73$\log_{10}$)**.

6. MRID 493674-08 "Standard Test Method for Efficacy of Sanitizers Recommended for Inanimate Non-Food Contact Surfaces (Dilutable), Test Organisms: *Campylobacter jejuni* (ATCC 29428) and *Pseudomonas aeruginosa* (ATCC 15442)"; by Kristen Niehaus. Study conducted at ATS Labs. Study completion date – July 24, 2014. Study Number A16710; Ecolab GLP Study Number: 1300150CL6.

This study was conducted against *Campylobacter jejuni* (ATCC 29428) and *Pseudomonas aeruginosa* (ATCC 15442). Two lots (Lot Nos. P111431-2 and P120331-2) of the product, KX-6228 (Synergex), were tested using the ATS Labs protocol ECO01050114.NFS.3 (copy provided). A use solution was prepared by adding 1.56 g test substance to 1498.44 g of 500 ppm AOAC synthetic hard water (titrated at 510 ppm; a 1 oz. per 8 gallons dilution). Fetal bovine serum was added to each inoculum to achieve a 5% organic soil load. Five sterile stainless steel carriers per product lot per organism were inoculated with 0.02 ml of a 48-54 hour old (*C. jejuni*) or adjusted (*P. aeruginosa)* suspension of the test organism. The inoculum was spread to within 1/8 inch of the edges of the carrier. The carriers were dried at 36.2°C and a relative humidity of 42%

(*C. jejuni*) or 27.5°C and a relative humidity of 65% (*P. aeruginosa)* for 40 minutes with the Petri dish lids intact. Each carrier was transferred to a sterile jar and was exposed to 5.0 ml of the use solution at 21.4°C and 43.3% relative humidity for 5 minutes. After exposure, 20 ml of D/E Neutralizing Broth was added to each jar and the jars were rotated vigorously to suspend the surviving organisms. Within 30 minutes of neutralization, duplicate 1.00 ml and 0.100 ml aliquots of the neutralized solution (10°) were plated onto the recovery agar plate medium. The *P. aeruginosa* plates were incubated at 35-37°C for 48±4 hours. The C. *jejuni* plates were incubated for 2-7 days at 35-37°C under microaerophilic conditions (CampyPak™ Plus). Following incubation the subcultures were visually enumerated. Controls included those for carrier quantitation, inoculum count, viability, neutralization confirmation, sterility, and purity. The reported average colony forming units (CFU) per control carrier, for each test microorganism, are as follows: ***Campylobacter jejuni* 6.03 x 10^6 (6.78$\log_{10}$) and *Pseudomonas aeruginosa* 1.86 x 10^5 (5.27$\log_{10}$)**.

7. MRID 493674-09 "Standard Test Method for Efficacy of Sanitizers Recommended for Inanimate Non-Food Contact Surfaces (Dilutable), Test Organisms: *Escherichia coli* O157:H7 (ATCC 35150), *Listeria monocytogenes* (ATCC 49594), and *Salmonella enterica* subsp. *enterica* Typhimurium (ATCC 13311)"; by Kristen Niehaus. Study conducted at ATS Labs. Study completion date – July 30, 2014. Study Number A16893; Ecolab GLP Study Number: 1300150CL7.

This study was conducted against *Escherichia coli* O157:H7 (ATCC 35150), *Listeria monocytogenes* (ATCC 49594), and *Salmonella enterica* subsp. *enterica* Typhimurium (ATCC 13311). Two lots (nos. P111431-2 and P120331-2) of the product, KX-6228 (Synergex), and two lots (nos. J072931 -2 and J113031-2) of Liquid K, were tested using the ATS Labs protocol ECO01052914.NFS (copy provided). Use solutions were prepared by adding 1.56g of KX-6228 Batch P111431-2 + 1496.43g 500 ppm AOAC synthetic hard water + 2.01 g of Liquid K Batch J072931-2 and 1.56g of KX-6228 Batch P120331-2 + 1496.43g 500 ppm AOAC synthetic hard water+ 2.01 g of Liquid K Batch J113031-2 (titrated at 489 ppm; a 1 oz. Synergex per 8 gallons plus 1 oz. Liquid K per 6 gallons). Fetal bovine serum was added to each inoculum to achieve a 5% organic soil load. Five sterile stainless steel carriers per product lot per organism were inoculated with 0.02 ml (*E. coli* and *S. enterica*) or 0.03 ml (*L. monocytogenes*) of a 48-54 hour old suspension of the test organism. The inoculum was spread to within 1/8 inch of the edges of the carrier. The carriers were dried at 36.0°C and a relative humidity of 40% with the Petri dish lids slightly ajar. Each carrier was transferred to a sterile jar and was exposed to 5.0 ml of the use solution at 22.09°C and 36.01% relative humidity for 5 minutes. After exposure, 20 ml of D/E Neutralizing Broth was added to each jar and the jars were rotated vigorously to suspend the surviving organisms. Within 30 minutes of neutralization, duplicate 1.00 ml and 0.100 ml aliquots of the neutralized solution (10°) were plated onto the recovery agar plate medium. The plates were incubated at 35-37°C for 48±4 hours. The subcultures were placed at 2-8°C for three days prior to examination. Following incubation and storage, the subcultures were visually enumerated. Controls included those for carrier quantitation, inoculum count, viability, neutralization confirmation, sterility, and purity. The reported average colony forming units (CFU) per control carrier, for each test microorganism, are as follows: ***Escherichia coli* O157:H7 1.38 x 10^6 (6.14$\log_{10}$), *Listeria monocytogenes* 2.19 x 10^5 (5.34$\log_{10}$), and *Salmonella enterica* subsp. *enterica* Typhimurium 2.24 x 10^5 (5.35$\log_{10}$)**.

Note: Protocol amendment and deviation reported in the study were reviewed

8. MRID 494674-10 "Germicidal and Detergent Sanitizing Action of Disinfectants, Test Organisms: *Vibrio cholerae* (ATCC 25873)"; by Matthew Sathe. Study conducted at ATS Labs. Study completion date – August 4, 2014. Study Number A16857; Ecolab GLP Study Number: 1300150CL8.

This study was conducted against *Vibrio cholerae* (ATCC 25873)**.** Two lots (Lot Nos. P111431-2 and P120331-2) of the product, KX-6228 (Synergex), were tested using the ATS Labs protocol ECO01061114.GDST (copy provided). A use solution was prepared by adding 1.19 g test substance to 998.81 g of 500 ppm AOAC synthetic hard water (titrated at 482 ppm; a 1 oz. per 7 gallons dilution). A 99-ml aliquot of the prepared use solution was transferred to each of 250-300 ml Erlenmeyer flasks. The flasks were placed in a 25±1°C water bath for at least 10 minutes. One-ml adjusted bacterial suspension was added to each flask. One-ml aliquots of the bacterium-test solution were transferred to 9 ml of Neutralizer 30 seconds after the addition of the bacterial suspension. After vortex mixing, four 1.0 ml and four 0.1 ml aliquots of the neutralized material were spread-plated onto the subculture agar medium. All subculture plates were incubated 24-30 hours at 35-37°C. Following incubation, the subculture plates were visually examined for growth. Controls included purity, sterility, viability, numbers control, and neutralization confirmation. The reported average initial colony forming units per ml, for each test microorganism, are as follows: ***Vibrio cholerae*** **(2.0×10^7, $7.30 \log_{10}$)**.

Note: Protocol amendment and deviation reported in the study were reviewed.

9. MRID 494674-11 "Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces, Test Organisms: 2009-H1N1 Influenza A virus (Novel H1N1) (Strain N Mexico/4108/2009, CDC #200971 2192)"; by Mary J. Miller. Study conducted at ATS Labs. Study completion date – August 12, 2014. Study Number A16974; Ecolab GLP Study Number: 1300150CL10.

This study was conducted against 2009-H1N1 Influenza A virus (Novel H1N1) (Strain N Mexico/4108/2009, CDC #200971 2192), using MDCK cells (canine kidney cells; propagated in-house; ATCC CCL-34) as the host system. Two lots (Lot Nos. P111431-2 and P120331-2) of the product, KX-6228 (Synergex), were tested according to ATS Labs Protocol No. ECO01062414.FLUA.2 (copy provided). A use solution was prepared by adding 1.19 g test substance to 998.81 g of 500 ppm AOAC synthetic hard water (titrated at 490 ppm; a 1 oz. per 7 gallons dilution).. The stock virus culture was adjusted to contain a 5% organic soil load (fetal bovine serum). Films of virus were prepared by spreading 0.2 ml of virus inoculum uniformly over the bottoms of separate sterile glass Petri dishes. The virus films were air-dried at 22.0°C for 20 minutes at 45% relative humidity. For each lot of product, separate dried virus films were individually exposed to a 2.00 mL aliquot of the use dilution of the test substance and held covered for 5 minutes at room temperature (20.0°C). After exposure, the plates were scraped with a cell scraper to re-suspend the contents. The virus-disinfectant mixture was passed through a Sephadex column, and diluted serially in Minimum Essential Medium (MEM) supplemented with 2 µg/mL TPCK-trypsin, 25 mM HEPES, 0.2% bovine serum albumin (BSA) fraction V, 10 µ/mL gentamicin, 100 units/mL penicillin, and 2.5 µg/mL amphotericin B. MDCK cells in multi-well culture dishes were inoculated in quadruplicate with 0.1 ml of the dilutions. The cultures were incubated at 36-38°C in a humidified atmosphere of 5-7% CO_2 and scored periodically for 7 days for the presence or absence of unspecified cytopathic effects, cytotoxicity, and viability. Controls included those for cytotoxicity, dried virus count, and neutralization. Viral and cytotoxicity titers were calculated by the method of Spearman Karber. The titer of the dried virus control was **7.00 $\log_{10}$**. Taking the cytotoxicity and neutralization control results into consideration, the reduction in viral titer was **6.50 $\log_{10}$** for both batches.

10. MRID 494674-12 "Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces, Test Organisms: Reovirus type 3, Strain Abney (ATCC VR-232)"; by Mary J. Miller. Study conducted at ATS Labs. Study completion date – August 12, 2014. Study Number A16972; Ecolab GLP Study Number: 1300150CL11.

This study was conducted against Reovirus type 3, Strain Abney (ATCC VR-232), using LLC-MK_2 cells (Rhesus monkey kidney cells; propagated in-house; ATCC CCL-7.1) as the host system. Two lots (Lot Nos. P111431-2 and P120331-2) of the product, KX-6228 (Synergex), were tested according to ATS Labs Protocol No. ECO01062414.REO (copy provided). A use solution was prepared by adding 1.19 g test substance to 998.81 g of 500 ppm AOAC synthetic hard water (titrated at 490 ppm; a 1 oz. per 7 gallons dilution).. The stock virus culture was adjusted to contain a 5% organic soil load (fetal bovine serum). Films of virus were prepared by spreading 0.2 ml of virus inoculum uniformly over the bottoms of separate sterile glass Petri dishes. The virus films were air-dried at 22.0°C for 20 minutes at 45% relative humidity. For each lot of product, separate dried virus films were individually exposed to a 2.00 mL aliquot of the use dilution of the test substance and held covered for 5 minutes at room temperature (20.0°C). After exposure, the plates were scraped with a cell scraper to re-suspend the contents. The virus-disinfectant mixture was passed through a Sephadex column, and diluted serially in Minimum Essential Medium (MEM) supplemented with 5% (v/v) heat-inactivated fetal bovine serum (FBS), 10 µ/mL gentamicin, 100 units/mL penicillin, and 2.5 µg/mL amphotericin B. LLC-MK_2 cells in multi-well culture dishes were inoculated in quadruplicate with 0.1 ml of the dilutions. The cultures were incubated at 36-38°C in a humidified atmosphere of 5-7% CO_2 and scored periodically for 7 days for the presence or absence of unspecified cytopathic effects, cytotoxicity, and viability. Controls included those for cytotoxicity, dried virus count, and neutralization. Viral and cytotoxicity titers were calculated by the method of Spearman Karber. The titer of the dried virus control was **6.50 $\log_{10}$**. Taking the cytotoxicity and neutralization control results into consideration, the reduction in viral titer was **6.0 $\log_{10}$** for both batches.

11. MRID 494674-13 "Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces, Test Organisms: **Influenza B virus, Strain B/Hong Kong/5/72 (ATCC VR-823)"; by Mary J. Miller. Study conducted at ATS Labs. Study completion date – August 12, 2014. Study Number A16975; Ecolab GLP Study Number: 1300150CL12.**

This study was conducted against Influenza B virus, Strain B/Hong Kong/5/72 (ATCC VR-823), using MDCK cells (canine kidney cells; propagated in-house; ATCC CCL-34) as the host system. Two lots (Lot Nos. P111431-2 and P120331-2) of the product, KX-6228 (Synergex), were tested according to ATS Labs Protocol No. ECO01062414.FLUB (copy provided). A use solution was prepared by adding 1.19 g test substance to 998.81 g of 500 ppm AOAC synthetic hard water (titrated at 490 ppm; a 1 oz. per 7 gallons dilution).. The stock virus culture was adjusted to contain a 5% organic soil load (fetal bovine serum). Films of virus were prepared by spreading 0.2 ml of virus inoculum uniformly over the bottoms of separate sterile glass Petri dishes. The virus films were air-dried at 22.0°C for 20 minutes at 45% relative humidity. For each lot of product, separate dried virus films were individually exposed to a 2.00 mL aliquot of the use dilution of the test substance and held covered for 5 minutes at room temperature (20.0°C). After exposure, the plates were scraped with a cell scraper to re-suspend the contents. The virus-disinfectant mixture was passed through a Sephadex column, and diluted serially in Minimum Essential Medium (MEM) supplemented with 2 µg/mL TPCK-trypsin, 25 mM HEPES, 0.2% bovine serum albumin (BSA) fraction V, 10 µ/mL gentamicin, 100 units/mL penicillin, and 2.5 µg/mL amphotericin B. MDCK cells in multi-well culture dishes were inoculated in quadruplicate with 0.1 ml of the

dilutions. The cultures were incubated at 36-38°C in a humidified atmosphere of 5-7% CO_2 and scored periodically for 7 days for the presence or absence of unspecified cytopathic effects, cytotoxicity, and viability. Controls included those for cytotoxicity, dried virus count, and neutralization. Viral and cytotoxicity titers were calculated by the method of Spearman Karber. The titer of the dried virus control was **5.75 $\log_{10}$**. Taking the cytotoxicity and neutralization control results into consideration, the reduction in viral titer was **5.25 $\log_{10}$** for both batches.

12. MRID 494674-14 "KX-6228 Food Contact Sanitizing Efficacy, Test Organisms: *Staphylococcus aureus* (ATCC 6538) and *Escherichia coli* (ATCC 11229)"; by Laurinda Holen. Study conducted at Ecolab. Study completion date – August 20, 2014. Study identification Number: 1400049.

This study was conducted against *Staphylococcus aureus* (ATCC 6538) and *Escherichia coli* (ATCC 11229). Three lots (Lot Nos. P081931-1, P111431-1 and P120331-1) of the product, KX-6228 (Synergex), were tested using Ecolab Microbiological Services (MS) Method MS009-25 (copy provided). A use solution was prepared by adding 1.19 g test substance to 998.81 g of 500 ppm AOAC synthetic hard water (titrated at 520 ppm; a 1 oz. per 7 gallons dilution). A 99-ml aliquot of the prepared use solution was transferred to each of 250 ml Erlenmeyer flasks. The flasks were placed in a 25±1°C water bath for at least 10 minutes. One-ml adjusted bacterial suspension was added to each flask. One-ml aliquots of the bacterium-test solution were transferred to 9 ml of Neutralizer 30 seconds after the addition of the bacterial suspension. After vortex mixing, four 1.0 ml and four 0.1 ml aliquots of the neutralized material were spread-plated onto the subculture agar medium. All subculture plates were incubated for 24-30 hours at 35±2°C. Following incubation, the subculture plates were visually examined for growth. Representative test and positive control subcultures showing growth were visually examined, Gram stained and biochemically assayed to confirm or rule out the presence of the test organism. Controls included purity, sterility, viability, numbers control, and neutralization confirmation. The reported average initial colony forming units per ml, for each test microorganism, are as follows: ***Staphylococcus aureus* 2.1 x 10^8 (9.32$\log_{10}$) and *Escherichia* coli 1.0 x 10^8 (8.01$\log_{10}$)**.

13. MRID 494674-15 "KX-6228 Non-Food Contact Sanitizing Efficacy, Test Organisms: *Staphylococcus aureus* (ATCC 6538) and *Enterobacter aerogenes* (ATCC 13048)"; by Laurinda Holen. Study conducted at Ecolab. Study completion date – July 8, 2014. Study identification Number: 1400050.

This study was conducted against *Staphylococcus aureus* (ATCC 6538) and *Enterobacter aerogenes* (ATCC 13048). Three lots (Lot Nos. P081931-1, P111431-1 and P120331-1) of the product, KX-6228 (Synergex), were tested using Ecolab Microbiological Services (MS) Method MS016-26 (copy provided). A use solution was prepared by adding ~1.56 g test substance to ~1498.44 g of 500 ppm AOAC synthetic hard water (titrated at 510 ppm; a 1 oz. per 8 gallons dilution). Fetal bovine serum was added to each inoculum to achieve a 5% organic soil load. Five sterile stainless steel carriers per product lot per organism were inoculated with 0.02 ml of a 48-54 hour old suspension of the test organism. The inoculum was spread to within 1/8 inch of the edges of the carrier. The carriers were dried at 35±2°C for 35±2 minutes with the Petri dish lids slightly ajar. Each carrier was transferred to a sterile jar and was exposed to 5.0 ml of the use solution at room temperature for 5 minutes. After exposure, 20 ml of D/E Neutralizing Broth was added to each jar and the jars were rotated vigorously to suspend the surviving organisms. Within 30 minutes of the addition of the neutralizer, 1.0 ml aliquots of the 10^0 dilutions were plated in duplicate on Tryptic Soy Agar with 5% Sheep Blood. Plates were incubated at 35±2°C (*S. aureus*) or 30±2°C (*E. aerogenes*) for 48±4 hours. All subcultures were placed at 2-8°C for 2 days prior to

examination. Following incubation and storage, the subcultures were visually enumerated. Controls included those for carrier quantitation, inoculum count, viability, neutralization confirmation, sterility, and purity. The reported average colony forming units (CFU) per control carrier, for each test microorganism, are as follows: ***Staphylococcus aureus*** **2.4 x 10^7 (7.38log_{10}) and *Enterobacter aerogenes* 1.7 x 10^7 (7.23log_{10})**.

14. MRID 494674-16 "KX-6228 Supplemental Food Contact Sanitizing Efficacy, Test Organisms: *Escherichia coli* O103:H6 (STEC), *Escherichia coli* O111:H8 (STEC), *Escherichia coli* O145:NM (STEC), *Escherichia coli* O26:H11 (STEC), *Escherichia coli* O45:H2 (STEC) and *Escherichia coli* O121:H19 (STEC)"; by Lisa Hellickson. Study conducted at Ecolab. Study completion date – August 8, 2014. Study identification Number: 1400060.

This study was conducted against *Escherichia coli* O103:H6 (STEC), *Escherichia coli* O111:H8 (STEC), *Escherichia coli* O145:NM (STEC), *Escherichia coli* O26:H11 (STEC), *Escherichia coli* O45:H2 (STEC) and *Escherichia coli* O121:H19 (STEC). Two lots (Lot Nos. P111431-1 and P120331-1) of the product, KX-6228 (Synergex), were tested using Ecolab Microbiological Services (MS) Method MS009-25 (copy provided). A use solution was prepared by adding 1.19 g test substance to 998.81 g of 500 ppm AOAC synthetic hard water (titrated at 500 and 520 ppm; a 1 oz. per 7 gallons dilution). A 99-ml aliquot of the prepared use solution was transferred to each of 250 ml Erlenmeyer flasks. The flasks were placed in a 25±1°C water bath for at least 10 minutes. One-ml adjusted bacterial suspension was added to each flask. One-ml aliquots of the bacterium-test solution were transferred to 9 ml of Neutralizer 30 seconds after the addition of the bacterial suspension. After vortex mixing, four 1.0 ml and four 0.1 ml aliquots of the neutralized material were spread-plated onto the subculture agar medium. All subculture plates were incubated for 24-30 hours at 35±2°C. The plates may have been stored at 2 - 8°C after incubation until the results were read. Following incubation and storage, the subculture plates were visually examined for growth. Controls included purity, sterility, viability, numbers control, and neutralization confirmation. The reported average initial colony forming units per ml, for each test microorganism, are as follows: ***Escherichia coli*** **O103:H6 6.2 x 10^7 (7.79log_{10}), *Escherichia coli* O111:H8 5.6 x 10^7 (7.75log_{10}), *Escherichia coli* O145:NM 3.9 x 10^7 (7.59log_{10}), *Escherichia coli* O26:H11 3.3 x 10^7 (7.52log_{10}), *Escherichia coli* O45:H2 6.5 x 10^7 (7.81log_{10}), and *Escherichia coli* O121:H19 6.0 x 10^7 (7.78log_{10})**.

Note: Protocol amendment and deviation reported in the study were reviewed

15. MRID 494674-17 "KX-6228 with Liquid K Foaming Non-Food Contact Sanitizing Efficacy, Test Organisms: *Staphylococcus aureus* (ATCC 6538) and *Enterobacter aerogenes* (ATCC 13048)"; by Laurinda Holen. Study conducted at Ecolab. Study completion date – August 27, 2014. Study identification Number: 1400067.

This study was conducted against *Staphylococcus aureus* (ATCC 6538) and *Enterobacter aerogenes* (ATCC 13048). Three lots (Lot Nos. P081931, P111431 and P120331) of the product, KX-6228 (Synergex) and three lots (nos. J071131, J072931 and J113031) of Liquid K, were tested using Ecolab Microbiological Services (MS) Method MS016-26 (copy provided). Use solutions were prepared by adding 1.59g of KX-6228 Batch P081931-1 + 1496.42g 500 ppm AOAC synthetic hard water + 2.01 g of Liquid K Batch J071131-1, 1.58g of KX-6228 Batch P111431-1 + 1496.43g 500 ppm AOAC synthetic hard water + 2.02 g of Liquid K Batch J072931-1, and 1.58g of KX-6228 Batch P120331-1 + 1496.43g 500 ppm AOAC synthetic hard water + 2.01 g of Liquid K Batch J113031-1 (titrated at 480 ppm and 520 ppm; a 1 oz. Synergex per 8 gallons plus 1 oz. Liquid K per 6 gallons). Fetal bovine serum was added to each inoculum to

achieve a 5% organic soil load. Five sterile stainless steel carriers per product lot per organism were inoculated with 0.02 ml of a 48-54 hour old suspension of the test organism. The inoculum was spread to within 1/8 inch of the edges of the carrier. The carriers were dried at 35±2°C for 35±2 minutes with the Petri dish lids slightly ajar. Each carrier was transferred to a sterile jar and was exposed to 5.0 ml of the use solution at room temperature for 5 minutes. After exposure, 20 ml of D/E Neutralizing Broth was added to each jar and the jars were rotated vigorously to suspend the surviving organisms. Within 30 minutes of the addition of the neutralizer, 1.0 ml aliquots of the 10^0 dilutions were plated in duplicate on Tryptic Soy Agar with 5% Sheep Blood. Plates were incubated at 35±2°C (*S. aureus*) or 30±2°C (*E. aerogenes*) for 48±4 hours. All subcultures were placed at 2-8°C for 2 days prior to examination. Following incubation and storage, the subcultures were visually enumerated. Controls included those for carrier quantitation, inoculum count, viability, neutralization confirmation, sterility, and purity. The reported average colony forming units (CFU) per control carrier, for each test microorganism, are as follows: ***Staphylococcus aureus* 2.4 x 10^7 (7.38$\log_{10}$) and *Enterobacter aerogenes* 1.7 x 10^7 (7.23$\log_{10}$)**.

16. MRID 494674-18 "KX-6228 Disinfection Efficacy, Test Organisms: *Staphylococcus aureus* (ATCC 6538) and *Salmonella enterica* (ATCC 10708)"; by Laurinda Holen. Study conducted at Ecolab. Study completion date – September 22, 2014. Study identification Number: 1400068.

This study was conducted against *Staphylococcus aureus* (ATCC 6538) and *Salmonella enterica* (ATCC 10708). Three lots (Lot Nos. P081931, P111431, and P120331) of the product, KX-6228 (Synergex), were tested using Ecolab Microbiological Services (MS) Method MS003-30 (copy provided). A use solution was prepared by adding 4.25 g or 4.16 g test substance to 1495.75 g or 1495.84g of 400 ppm or 500 ppm AOAC synthetic hard water (titrated at 410 ppm, 490 ppm, or 510 ppm; a 1 oz. per 3 gallons dilution). Fetal bovine serum was added to the culture to achieve a 5% organic soil load. Sixty (60) polished stainless steel penicillin cup carriers were immersed for 15±2 minutes in a 48-54 hour old suspension of the test organism, at a ratio of 1 carrier per 1.0 ml broth. The carriers were dried for 40±2 minutes at 35±2°C. Each carrier was exposed to 10 ml of the use solution for 10 minutes at 20±1°C. After exposure, the carriers were transferred to 10 ml of Letheen Broth containing 0.5% Sodium Thiosulfate to neutralize. All subcultures were incubated for 48±2 hours at 35±2°C. Following incubation, the subcultures were examined for the presence or absence of visible growth. Controls included those for purity, sterility, viability, neutralization confirmation, and carrier population.

Note: Protocol amendments reported in the study were reviewed

17. MRID 494674-19 "AOAC Use-Dilution Method, Test Organisms: *Pseudomonas aeruginosa* (ATCC 15442)"; by Kristen Niehaus. Study conducted at ATS Labs. Study completion date – September 22, 2014. Project Number A17044; Ecolab GLP Study Number: 1400084CL1.

This study was conducted against *Pseudomonas aeruginosa* (ATCC 15442). Three lots (Lot Nos. P072241-CL, P111431–CL, and P120331-CL) of the product, KX-6228 (Synergex), were tested using ATS Labs protocol no. ECO01062414.UD (copy provided). A use solution was prepared by adding 2.66 g or 2.77 g test substance to 997.34 g or 997.23g of 500 ppm AOAC synthetic hard water (titrated at 500 ppm or 503 ppm; a 1 oz. per 3 gallons dilution). Fetal bovine serum was added to the culture to achieve a 5% organic soil load. Sixty (60) stainless steel penicylinders carriers were immersed for 15±2 minutes in a 48-54 hour old suspension of the test organism, at a ratio of 1 carrier per 1.0 ml broth. The carriers were dried for 38 minutes at 35.5-36.3°C and 54.8-55.5% relative humidity. Each carrier was exposed to 10 ml of the use solution for 10 minutes at 19-20°C. After exposure, the carriers were transferred to 10 ml of Letheen Broth containing

0.5% Sodium Thiosulfate to neutralize. All subcultures were incubated for 48±2 hours at 35-37°C. For testing performed on 8/8/14, subcultures were stored at 2-8°C for one day prior to examination. Following incubation and storage, the subcultures were visually examined for the presence or absence of growth. On 8/11/14, 8/1 3/14 and 8/28/14, representative test and positive control subculture tubes showing growth were subcultured to Tryptic Soy Agar + 5% Sheep's blood and incubated at 35-37°C for one day. The resultant growth was visually examined, Gram stained and biochemically assayed to confirm or rule out the presence of the test organism. Controls included those for purity, sterility, viability, neutralization confirmation, and carrier population.

Note: Protocol amendments reported in the study were reviewed

Note: The applicant provided the data for two failed trials. In those trial, the numbers controls were below the required number. Thus, the tests were invalid. These data were not used to evaluate efficacy of the test product. See Attachment I of the laboratory report.

18. MRID 494674-20 "Fungicidal Use-Dilution Method, Test Organisms: *Trichophyton mentagrophytes* (ATCC 9533)"; by Kristen Niehaus. Study conducted at ATS Labs. Study completion date – September 30, 2014. Project Number A17097; Ecolab GLP Study Number: 1400084CL3.

This study was conducted against *Trichophyton mentagrophytes* (ATCC 9533). Two lots (Lot Nos. P111431–C, and P120331-CL) of the product, KX-6228 (Synergex), were tested using ATS Labs protocol no. ECO01062414.FUD.1 (copy provided). A use solution was prepared by adding 1.54 g or 1.51 g test substance to 998.49 g or 998.51g of 500 ppm AOAC synthetic hard water (titrated at 473 ppm or 503 ppm; a 1 oz. per 5.5 gallons dilution). Fetal bovine serum was added to the culture to achieve a 5% organic soil load. Sixty (60) stainless steel penicylinders carriers were immersed for 15±2 minutes in an adjusted solution of conidia, at a ratio of 1 carrier per 1.0 ml. The carriers were dried for 38 minutes at 35.5-36.0°C and 40% relative humidity. Each carrier was exposed to 10 ml of the use solution for 10 minutes at 20°C. After exposure, the carriers were transferred to 10 ml of Sabouraud Dextrose Broth + 0.07% Lecithin + 0.5% Tween 80 to neutralize. All neutralized subcultures were incubated for 10 days at 25-30°C. The agar plate subcultures were incubated for 44-76 hours at 25-30°C. Agar plate subcultures were stored at 2-8°C for two days prior to examination. Neutralized subcultures were stored at 2-8°C for one day prior to examination. Following incubation and storage, the subcultures were visually examined for the presence or absence of visible growth. Controls included those for purity, sterility, viability, neutralization confirmation, and carrier population.

V. RESULTS

MRID #	Organism	Lot #	Results-1oz/7gal-(CFU/ml)		Log Reduction
			Survivors	Controls	
494674-03	*Escherichia* coli O157:H7	P111431-2	<1	3.0×10^7	>7.48
		P120331-2	<1		>7.48
	Listeria monocytogenes	P111431-2	9	7.9×10^7	6.95
		P120331-2	4×10^1		6.30
	Salmonella enterica	P111431-2	9	9.5×10^7	7.03
		P120331-2	<1		>7.98
494674-04		P111431-2	<1	7.9×10^7	>7.87

	Campylobacter jejuni	P120331-2	<1		>7.87
494674-05	*Cronobacter sakazakii*	P111431-2	<1	4.9×10^7	>7.69
		P120331-2	<1		>7.69
	Pseudomonas aeruginosa	P111431-2	<1	3.9×10^7	>7.59
		P120331-2	<1		>7.59
494674-10	*Vibrio cholerae*	P111431-2	<1	2.0×10^7	>7.30
		P120331-2	<1		>7.30
494674-14	*Staphylococcus aureus* (ATCC 6538)	P081931-1	$<1.8 \times 10^1$	2.1×10^8	>7.06
		P111431-1	$<1.8 \times 10^1$		>7.06
		P120331-1	$<1.8 \times 10^1$		>7.06
	Escherichia coli (ATCC 11229)	P081931-1	$<1.8 \times 10^1$	1.0×10^8	>6.75
		P111431-1	$<1.8 \times 10^1$		>6.75
		P120331-1	$<1.8 \times 10^1$		>6.75
494674-16	*Escherichia coli* O103:H6	P111431-1	$<1.8 \times 10^1$	6.2×10^7	>6.53
		P120331-1	$<1.8 \times 10^1$		>6.53
	Escherichia coli O111:H8	P111431-1	$<1.8 \times 10^1$	5.6×10^7	>6.49
		P120331-1	$<1.8 \times 10^1$		>6.49
	Escherichia coli O145:NM	P111431-1	$<1.8 \times 10^1$	3.9×10^7	>6.33
		P120331-1	$<1.8 \times 10^1$		>6.33
	Escherichia coli O26:H11	P111431-1	$<1.8 \times 10^1$	3.3×10^7	>6.26
		P120331-1	$<1.8 \times 10^1$		>6.26
	Escherichia coli O45:H2	P111431-1	$<1.8 \times 10^1$	6.5×10^7	>6.55
		P120331-1	$<1.8 \times 10^1$		>6.55
	Escherichia coli O121:H19	P111431-1	$<1.8 \times 10^1$	6.0×10^7	>6.52
		P120331-1	$<1.8 \times 10^1$		>6.52

MRID #	Organism	Lot #	Results-1oz/8gal-(CFU/carrier)		Percent Reduction
			Survivors	Controls	
494674-06	*Escherichia* coli O157:H7	P111431-2	$<2.51 \times 10^1$	3.24×10^6	>99.9%
		P120331-2	$<2.51 \times 10^1$		>99.9%
	Listeria monocytogenes	P111431-CL	$<2.13 \times 10^2$	1.78×10^6	>99.9%
		P120331-CL	$<2.51 \times 10^1$		>99.9%
494674-07	*Cronobacter sakazakii*	P111431-2	$<2.51 \times 10^1$	6.03×10^4	>99.9%
		P120331-2	$<2.51 \times 10^1$		>99.9%
	Salmonella enterica	P111431-2	$<2.51 \times 10^1$	5.37×10^5	>99.9%
		P120331-2	$<2.51 \times 10^1$		>99.9%
494674-08	*Campylobacter jejuni*	P111431-2	$<2.51 \times 10^1$	6.03×10^6	>99.9%
		P120331-2	$<2.51 \times 10^1$		>99.9%
	Pseudomonas aeruginosa	P111431-2	$<2.51 \times 10^1$	1.86×10^5	>99.9%
		P120331-2	$<2.51 \times 10^1$		>99.9%
494674-09 Plus 1oz./6gal Liquid K Foaming	*Escherichia coli* O157:H7	P111431-2	$<2.51 \times 10^1$	1.38×10^6	>99.9%
		P120331-2	$<2.51 \times 10^1$		>99.9%
	Listeria monocytogenes	P111431-2	$<1.17 \times 10^2$	2.19×10^5	>99.9%
		P120331-2	$<5.62 \times 10^1$		>99.9%
	Salmonella enterica	P111431-2	$<2.51 \times 10^1$	2.24×10^5	>99.9%
		P120331-2	$<2.51 \times 10^1$		>99.9%

494674-15	*Staphylococcus aureus* (ATCC 6538)	P081931-1	<2.5 x 10^1	2.4 x 10^7	>99.9%
		P111431-1	<2.5 x 10^1		>99.9%
		P120331-1	<2.7 x 10^1		>99.9%
	Enterobacter aerogenes (ATCC 13048)	P081931-1	<9.2 x 10^1	1.7 x 10^7	>99.9%
		P111431-1	<8.4 x 10^1		>99.9%
		P120331-1	<1.2 x 10^2		>99.9%
494674-17 Plus 1oz./6gal Liquid K Foaming	*Staphylococcus aureus* (ATCC 6538)	P081931-1	<2.5 x 10^1	2.5 x 10^7	>99.9%
		P111431-1	<5.4 x 10^1		>99.9%
		P120331-1	<3.5 x 10^1		>99.9%
	Enterobacter aerogenes (ATCC 13048)	P081931-1	<3.0 x 10^1	2.7 x 10^7	>99.9%
		P111431-1	<3.6 x 10^1		>99.9%
		P120331-1	<3.7 x 10^2		>99.9%

MRID #	**Organism**	**Results – 1oz/7gal – 5 minutes**			**Dried Virus Control ($TCID_{50}$/0.1 ml)**
			Lot No. P111431-2	**Lot No. P120331-2**	
494674-11	2009-H1N1 Influenza A virus	10^{-1} to 10^{-8} dilutions	Complete inactivation	Complete inactivation	$10^{7.00}$
		$TCID_{50}$/0.1 ml	$\leq 10^{0.50}$	$\leq 10^{0.50}$	
		TCD_{50}/0.1 ml	$\leq 10^{0.50}$	$\leq 10^{0.50}$	
		Log reduction	≥6.50	≥6.50	
494674-12	Reovirus type 3	10^{-1} to 10^{-8} dilutions	Complete inactivation	Complete inactivation	$10^{6.50}$
		$TCID_{50}$/0.1 ml	$\leq 10^{0.50}$	$\leq 10^{0.50}$	
		TCD_{50}/0.1 ml	$\leq 10^{0.50}$	$\leq 10^{0.50}$	
		Log reduction	≥6.00	≥6.00	
494674-12	Influenza B virus	10^{-1} to 10^{-8} dilutions	Complete inactivation	Complete inactivation	$10^{5.75}$
		$TCID_{50}$/0.1 ml	$\leq 10^{0.50}$	$\leq 10^{0.50}$	
		TCD_{50}/0.1 ml	$\leq 10^{0.50}$	$\leq 10^{0.50}$	
		Log reduction	≥5.25	≥5.25	

MRID Number	**Organism**	**Contact Time**	**No. Exhibiting Growth/Total No. Tested - 1oz/3gallons**			**Dried Carrier Count (Log_{10})**
			Lot No. P081931	**Lot No. P111431**	**Lot No. P120331**	
	Staphylococcus aureus		0/60 1/60	1/60	0/60	6.26 - 6.11 - 6.48 -6.34

494674-18	*Salmonella choleraesuis*	10 minutes	1/60 0/60	1/60	0/60	6.76 - 5.79 - 5.87 - 5.70
			Lot No. P072241-CL	**Lot No. P111431-CL**	**Lot No. P120331-CL**	
494674-19	*Pseudomonas aeruginosa*		2/60	2/60	1/60	6.08 - 6.26 – 6.88
			1 oz. per 5.5 gallons			
494674-20	*Trichophyton mentagrophytes*		--	0/10	0/10	5.16

VI. CONCLUSION

1. The submitted efficacy data **support** the use of the product, Synergex (EPA File Symbol 1677-ELN), as a sanitizer for pre-cleaned food contact surfaces, against the following microorganisms when used at 1 oz. per 7 gallons of 500 ppm hard water, for 30-second contact time at room temperature:

Escherichia coli O157:H7 (ATCC 35150)	MRID 494674-03
Listeria monocytogenes (ATCC 49594)	MRID 494674-03
Salmonella enterica subsp. *enterica* Typhimurium (ATCC 13311)	MRID 494674-03
Campylobacter jejuni (ATCC 29428)	MRID 494674-04
Cronobacter sakazakii (ATCC 12868)	MRID 494674-05
Pseudomonas aeruginosa (ATCC 15442)	MRID 494674-05
Vibrio cholerae (ATCC 25873)	MRID 494674-10
Staphylococcus aureus (ATCC 6538)	MRID 494674-14
Escherichia coli (ATCC 11229)	MRID 494674-14
Escherichia coli O103:H6 (STEC)	MRID 494674-16
Escherichia coli O111:H8 (STEC)	MRID 494674-16
Escherichia coli O145:NM (STEC)	MRID 494674-16
Escherichia coli O26:H11 (STEC)	MRID 494674-16
Escherichia coli O45:H2 (STEC)	MRID 494674-16
Escherichia coli O121:H19 (STEC)	MRID 494674-16

2. The submitted efficacy data **support** the use of the product, Synergex (EPA File Symbol 1677-ELN), as a sanitizer against the following microorganisms on hard, nonporous surfaces in the presence of a 5% organic soil load, when used at 1 oz. per 8 gallons of 500 ppm hard water, for a 5-minute contact time, at room temperature:

Escherichia coli O157:H7 (ATCC 35150)	MRID 494674-06
Listeria monocytogenes ATCC 49594)	MRID 494674-05
Cronobacter sakazakii (ATCC 12868)	MRID 494674-07
Salmonella enterica subsp. *enterica* Typhimurium (ATCC 13311)	MRID 494674-07
Campylobacter jejuni (ATCC 29428)	MRID 494674-08
Pseudomonas aeruginosa (ATCC 15442)	MRID 494674-08
Staphylococcus aureus (ATCC 6538)	MRID 494674-15
Enterobacter aerogenes (ATCC 13048)	MRID 494674-15

3. The submitted efficacy data **support** the use of the product, Synergex (EPA File Symbol 1677-ELN), as a sanitizer against the following microorganisms on hard, nonporous surfaces in the presence of a 5% organic soil load, when used at 1 oz. Synergex per 8 gallons plus 1 oz. Liquid K Foaming per 6 gallons, in 500 ppm hard water, for a 5-minute contact time, at room temperature:

Escherichia coli O157:H7 (ATCC 35150)	MRID 494674-09
Listeria monocytogenes (ATCC 49594)	MRID 494674-09
Salmonella enterica subsp. *enterica* Typhimurium (ATCC 13311)	MRID 494674-09
Staphylococcus aureus (ATCC 6538)	MRID 494674-15
Enterobacter aerogenes (ATCC 13048)	MRID 494674-15

4. The submitted efficacy data **support** the use of the product, Synergex (EPA File Symbol 1677-ELN), as a disinfectant with virucidal activities against the following microorganisms on hard, nonporous surfaces in the presence of a 5% organic soil load, when used at 1 oz. per 7 gallons of 500 ppm hard water, for a 5-minute contact time, at room temperature:

2009-H1N1 Influenza A virus (Novel H1N1), Strain N Mexico/4108/2009, (CDC #2009712192)	MRID 494674-11
Reovirus type 3, Strain Abney (ATCC VR-232)	MRID 494674-12
Influenza B virus, Strain B/Hong Kong/5/72 (ATCC VR-823)	MRID 494674-13

5. The submitted efficacy data **support** the use of the product, Synergex (EPA File Symbol 1677-ELN), as a disinfectant with bactericidal activities against the following microorganisms on hard, nonporous surfaces in the presence of a 5% organic soil load, when used at 1 oz. per 3 gallons of 500 ppm hard water, for a 10-minute contact time, at room temperature:

Staphylococcus aureus (ATCC 6538)	MRID 494674-18
Salmonella enterica (ATCC 10708)	MRID 494674-18
Pseudomonas aeruginosa (ATCC 15442)	MRID 494674-19

6. The submitted efficacy data **support** the use of the product, Synergex (EPA File Symbol 1677-ELN), as a disinfectant with fungicidal activities against *Trichophyton mentagrophytes* (ATCC 9533) on hard, nonporous surfaces in the presence of a 5% organic soil load, when used at 1 oz. per 5.5 gallons of 500 ppm hard water, for a 10-minute contact time, at room temperature.

VII. LABEL

1. The proposed label claims **are acceptable** regarding the use of the product, Synergex (EPA File Symbol 1677-ELN), as a pre-cleaned food contact surfaces sanitizer against the following microorganisms when used at 1 oz. per 7 gallons of 500 ppm hard water, for at least 1 minute, at room temperature:

Escherichia coli O157:H7 (ATCC 35150)
Listeria monocytogenes (ATCC 49594)
Salmonella enterica subsp. *enterica* Typhimurium (ATCC 13311)
Campylobacter jejuni (ATCC 29428)
Cronobacter sakazakii (ATCC 12868)
Pseudomonas aeruginosa (ATCC 15442)

Vibrio cholerae (ATCC 25873)
Staphylococcus aureus (ATCC 6538)
Escherichia coli (ATCC 11229)
Escherichia coli O103:H6 (STEC)
Escherichia coli O111:H8 (STEC)
Escherichia coli O145:NM (STEC)
Escherichia coli O26:H11 (STEC)
Escherichia coli O45:H2 (STEC)
Escherichia coli O121:H19 (STEC)

These claims **are supported** by the applicant's data.

2. The proposed label claims **are acceptable** regarding the use of the product, Synergex (EPA File Symbol 1677-ELN), as a sanitizer against the following microorganisms on hard, nonporous surfaces in the presence of a 5% organic soil load, when used at 1 oz. per 8 gallons of 500 ppm hard water for a 5-minute contact time at room temperature:

Escherichia coli O157:H7 (ATCC 35150)
Listeria monocytogenes (ATCC 49594)
Cronobacter sakazakii (ATCC 12868)
Salmonella enterica subsp. *enterica* Typhimurium (ATCC 13311)
Campylobacter jejuni (ATCC 29428)
Pseudomonas aeruginosa (ATCC 15442)
Staphylococcus aureus (ATCC 6538)
Enterobacter aerogenes (ATCC 13048)

These claims **are supported** by the applicant's data.

3. The proposed label claims **are acceptable** regarding the use of the product, Synergex (EPA File Symbol 1677-ELN), as a sanitizer against the following microorganisms on hard, nonporous surfaces in the presence of a 5% organic soil load, when used at 1 oz. Synergex per 8 gallons plus 1 oz. Liquid K Foaming per 6 gallons, in 500 ppm hard water, for a 5-minute contact time, at room temperature:

Escherichia coli O157:H7 (ATCC 35150),
Listeria monocytogenes (ATCC 49594)
Salmonella enterica subsp. *enterica* Typhimurium (ATCC 13311)
Staphylococcus aureus (ATCC 6538)
Enterobacter aerogenes (ATCC 13048)

These claims **are supported** by the applicant's data.

4. The proposed label claims **are acceptable** regarding the use of the product, Synergex (EPA File Symbol 1677-ELN), as a disinfectant with virucidal activities against the following microorganisms on hard, nonporous surfaces in the presence of a 5% organic soil load, when used at 1 oz. per 7 gallons of 500 ppm hard water for a 5-minute contact time at room temperature:

2009-H1N1 Influenza A virus (Novel H1N1), Strain A/Mexico/4108/2009, (CDC #2009712192)
Reovirus type 3, Strain Abney (ATCC VR-232)
Influenza B virus, Strain B/Hong Kong/5/72 (ATCC VR-823)

These claims **are supported** by the applicant's data.

5. The proposed label claims **are acceptable** regarding the use of the product, Synergex (EPA File Symbol 1677-ELN), as a disinfectant with bactericidal activities against the following microorganisms on hard, nonporous surfaces in the presence of a 5% organic soil load, when used at 1 oz. per 3 gallons of 500 ppm hard water for a 10-minute contact time at room temperature:

Staphylococcus aureus (ATCC 6538)
Salmonella enterica (ATCC 10708)
Pseudomonas aeruginosa (ATCC 15442)

These claims **are supported** by the applicant's data.

6. The proposed label claims **are acceptable** regarding the use of the product, Synergex (EPA File Symbol 1677-ELN), as a disinfectant with fungicidal activities against *Trichophyton mentagrophytes* (ATCC 9533), on hard, nonporous surfaces in the presence of a 5% organic soil load, when used at 1 oz. per 5.5 gallons of 500 ppm hard water for a 5-minute contact time at room temperature. These claims **are supported** by the applicant's data.

7. The proposed label claims **are not acceptable** regarding the use of the product, Synergex (EPA File Symbol 1677-ELN), as an "Antimicrobial Rinse For Pre-Cleaned Food Contact Surfaces" against *Geobacillus stearothermophilus* (ATCC 7953), when used at 1 oz. per 7 gallons for a 5-minute contact time at 50°C to 60°C. **The product failed to reduce at least 3log$_{10}$ spores of the microorganisms within the specified conditions. Claims for *Geobacillus stearothermophilus* (ATCC 7953) must be removed from the label.**